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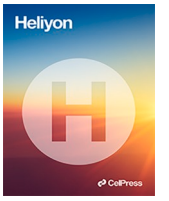

Review article

Point-of-care testing for early-stage liver cancer diagnosis and personalized medicine: Biomarkers, current technologies and perspectives

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ABSTRACT

Liver cancer is a highly prevalent and lethal form of cancer worldwide. In the absence of early diagnosis, treatment options for this disease are severely restricted. Recent advancements in genomics and bioinformatics have facilitated the discovery of a multitude of novel biomarkers that accurately depict an individual's disease diagnosis, progression, and treatment response. Leveraging these breakthroughs, personalized medicine employs an individual's biomarker profile to enable early detection of liver cancer and inform decisions regarding treatment selection, dosage determination, and prognosis assessment. The current lack of readily applicable, timely, and economically viable tools for biomarker analysis has hindered the incorporation of personalized medicine into regular clinical procedures. Over the past decade, significant advancements have been achieved in the field of molecular point-of-care testing (POCT) and amplification techniques, leading to substantial improvements in the diagnosis of liver cancer and the implementation of precision medicine. Instrument-free PCR technology or plasma PCR technology can shorten the complex procedure of in vitro detection of nucleic acid-based biomarkers. Also, compared to traditional ELISA, various nanomaterials modified with monoclonal antibodies to target proteins for recognition, capture, and detection have improved the efficiency of protein-based biomarker detection. These advances have reduced the time and cost of clinical detection of early-stage hepatocellular carcinoma and improved the efficiency of timely diagnosis and survival of suspected patients while reducing unnecessary testing costs and procedures. This review aims to provide a comprehensive overview of the current and emerging biomarkers employed in the early detection of liver cancer, as well as the advancements in point-of-care molecular testing technology and platforms. The primary objective is to assess their potential in facilitating the implementation of personalized medicine. This review ultimately revealed that the diagnosis of early-stage hepatocellular carcinoma not only requires sensitive biomarkers, but its various modifications and changes during the progression of cirrhosis to early-stage hepatocellular carcinoma will be a greater focus of our attention in the future. The rapid development of POCT has facilitated the opportunity to readily detect liver cancer in the general population in the future, and the integration of multi-pathway multiplexing and intelligent algorithms has improved the sensitivity and accuracy of early liver cancer biomarker detection. It is expected

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that the integration of point-of-care technology will be instrumental in the widespread adoption of personalized medicine in the foreseeable future.

Abbreviations:

HCC	hepatocellular carcinoma
AASLD	American Association for the Study of Liver Diseases
HBV	hepatitis B virus
POCT	point of care testing
AFP	alpha-fetoprotein
AFP-L3	lens culinaris agglutinin-reactive fraction of AFP
DCP	des-gamma carboxyprothrombin
ANHC	AFP-negative hepatocellular carcinoma
EVs	extracellular vesicles
TIF	tumor interstitial fluid
ECM	extracellular matrix
PCR	polymerase chain reaction
cfDNA	circulating free DNA
ctDNA	circulating tumor DNA
DMB	differential methylation block
CTCs	circulating tumor cells
lcWGS	low-coverage whole genome sequencing
SCNA	somatic copy number aberrations
tsRNA	tRNA-derived small RNA
circRNA	circular RNA
lncRNA	long non-coding RNA
qRT-PCR	quantitative reverse transcription polymerase chain reaction
LAMP	loop-mediated isothermal amplification
ITA	isothermal amplification technique
Hsp90α	heat shock protein 90alpha
TK1	thymidine kinase 1
NAFLD	non-alcoholic fatty liver disease
LCN-2	lipocalin 2
MMP-9	matrix metallopeptidase 9
CSC	cancer stem cells
CAFs	cancer-associated fibroblasts
HSCs	hepatic stellate cells
FGF	fibroblast growth factor
VEGF	vascular endothelial growth factor
TME	tumor microenvironment
EMT	epithelial-mesenchymal transition
MET	mesenchymal-epithelial transition
PET	positron emission tomography
AAb	autoantibodies
TAA	tumors associated antigens
ANN	artificial neural network
ELISA	enzyme-linked immunosorbent assay
EpCAM	epithelial cell adhesion molecule
CSV	cell surface vimentin
TGF-β/sirtuin	transforming growth factor beta/sirtuin
MS	mass spectrometry
UPLC	ultra-high-performance liquid chromatography
HPLC	high-performance liquid chromatography
COX-2	cyclooxygenase-2
CI	confidence interval
DNBA	3,5-dinitrobenzoic acid
EDN	entropy-driven dynamic DNA network
GO	graphene oxide;

Dz	DNAzyme
LOD	limit of detection
AuNFs	Au nanoflowers
PNA	peptide nucleic acids
CFME	carbon fiber microelectrode
WE	working electrode
3D-GA	three-dimensional graphene aerogel
EIS	electrochemical impedance spectroscopy
LED	light-emitting diode
CHA	catalytic hairpin assembly
hMFEX	homogeneous magneto fluorescent exosome
SPR	surface plasmon resonance
t-SPR	transport surface plasmon resonance
SERS	Surface-enhanced Raman spectroscopy
MIPs	molecularly imprinted polymers
AAO	anodic aluminum oxide;
DL:	deep learning
BLI	bioluminescence imaging
GFP	green fluorescent protein
BLT	bioluminescence tomography
BRET	bioluminescence resonance energy transfer
MPs	magnetic microparticles
μPADs	microfluidic paper-based analytical devices
PUA	polyurethane acrylate
NC	nitrocellulose
ML:	machine algorithms
FS	feature selection
DNN	deep neural network
Au NPs	gold nanoparticles
NPs	novel nanoparticles
TL:	detection lines
CL:	control lines
PPG	photoelectric volumetric pulse wave
MSS	multichannel smartphone spectrometer
LRS	low resource setting
FR	folate receptor
TBil	total bilirubin
DBil	direct bilirubin
ALS	ambient light sensor

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1. Introduction

1.1. Overall trends of primary liver cancer

Primary liver cancer is a prevalent malignancy worldwide, with increasing incidence and mortality rates [1,2]. In April 2024, the International Agency for Research on Cancer (IARC) reported that in 2020, over one million individuals succumbed to liver cancer, establishing it as the third foremost cause of cancer-related fatalities globally [3]. In 2023, the United States recorded 41,210 new instances of liver cancer [4]. It accounts for a significant number of new cancer cases and fatalities each year, ranking as the sixth most commonly diagnosed cancer and the fourth largest cause of cancer-related mortality [5–7]. Hepatocellular carcinoma (HCC) represents the predominant type of primary liver cancer [8]. By 2020, there will be more than 900,000 new cases of liver cancer globally, with HCC constituting 75–85 % of cases and ICC accounting for 10–15 %. The incidence of ICC is increasing, although ambiguous symptoms and the absence of effective early screening tools characterize it. HCC is the predominant form of primary liver cancer and a significant area of research emphasis. The contemporary lifestyle of the youth, characterized by insufficient sleep, nocturnal activities, and alcohol consumption, may elevate the risk of hepatitis, cirrhosis, and early-stage HCC [7]. Fewer than 20 % of individuals qualify for curative treatment owing to advanced tumor stage [9]. The advocacy for neonatal hepatitis B vaccine and aflatoxin monitoring has diminished the prevalence of HCC. However, alcohol consumption, along with the emergence of obesity and diabetes, increases the risk of developing alcoholic or nonalcoholic steatohepatitis (NAFLD), thus aggravating the advancement from alcoholic and metabolic liver disease to HCC [10]. Thus, the prevention and detection of liver cancer predominantly focus on managing HCC.

1.2. Early detection of liver cancer

Early-stage HCC often manifests asymptomatically, rendering it susceptible to being overlooked. Patients frequently obtain a diagnosis when the disease has progressed to middle or advanced stages [11]. Furthermore, the prognosis for HCC is poor, marked by a low survival rate, underscoring the vital necessity of early identification as the only means to enhance survival outcomes [12]. Currently, research predominantly concentrates on enhancing the efficacy of early HCC detection. Cancer biomarkers are essential for detecting early-stage malignancies and evaluating later recurrences after treatment [13].

According to the 2023 guidelines set forth by the American Association for the Study of Liver Diseases (AASLD), the utilization of biomarkers, specifically AFP alone, for diagnosing early-stage HCC is discouraged due to its inadequate detection accuracy [14]. Additionally, non-invasive imaging is not recommended for chronic hepatitis B virus (HBV) patients lacking cirrhosis or exhibiting a low risk of HCC. This is chiefly attributable to the insufficient precision observed in these patient cohorts. Consequently, it is advisable to utilize both ultrasound and AFP monitoring concurrently as an alternative strategy for the prompt detection of liver cancer. Thus, the early detection of liver cancer necessitates the advancement of innovative detection platforms and techniques, as well as the exploration of novel biomarkers specifically targeting early-stage liver cancer. Notwithstanding its intrinsic limitations, AFP remains a significant biomarker for the prompt detection of HCC, although it fails to detect HCC in approximately 30 %–40 % of patients with normal AFP levels [15]. Presently, the absence of efficacious biomarkers for early HCC renders the treatment of this ailment inadequate [16]. The limited abundance of specific biomarkers often constrains the development of analytical tools for early-stage disease diagnosis [17].

1.3. POCT for early cancer detection and precision medicine

Recent advancements in point-of-care testing (POCT), a burgeoning diagnostic platform, have significantly enhanced the management and monitoring of diseases on-site. POCT denotes tests conducted in proximity to the patient using advanced detection technology [18]. These tests entail direct reactions with the specimen employing dry chemistry and immunolabelling techniques, yielding colorimetric alterations or instrumental evaluations for diagnosis. POCT can integrate biosensors, biochips, and intelligent algorithms to enhance diagnostic precision and portability for quantitative and automated assessments [19]. The implementation of POCT has facilitated the prompt detection of cancer. POCT entails performing tests at the location of sample collection with portable analytical instruments and associated reagents, facilitating the immediate retrieval of test outcomes. POCT, initially utilized in blood glucose meters, blood pressure meters, and pregnancy test strips, has garnered widespread recognition due to its promptness, convenience, and precision. Advancements in contemporary electrochemistry, fluorescence detection technology, and integrating multi-pathway platforms such as digital microfluidics have facilitated the application of innovative POCT devices to detect emerging early HCC biomarkers in patients [20]. This could significantly improve detection efficiency, allowing patients to conveniently and promptly comprehend their physiological situation and increase their chances of survival.

Despite the existence of significant review papers that summarise advancements in POCT technology [21] and its utilization in cancer diagnostics [22], there is a deficiency of review papers that specifically concentrate on biomarkers and POCT strategies for the early detection of HCC and precision medicine. Consequently, there is a pressing need for a timely review to address this gap. This paper examines the latest biomarkers for early-stage HCC and advancements in POCT technology and platforms. Additionally, we provide a succinct summary of the potential and obstacles associated with techniques for early liver cancer detection and precision medicine.

2. Methodology

The review focused on using keywords like "point-of-care testing," "biomarker," "early-stage cancer detection," "hepatocellular carcinoma diagnostics," and "personalized medicine" in a literature search from databases like PubMed and Web of Science. It specifically looked at new biomarkers for early liver cancer detection and the use of POCT in clinical settings. We carefully selected recent articles from the last decade and focused on research papers from 2020 to 2023 on POCT detection of early liver cancer.

3. Biomarkers for early liver cancer

Utilizing uncomplicated and effective diagnostic techniques is imperative for the timely identification and management of liver cancer. Early-stage HCC denotes cancer cells that have not infiltrated hepatic vessels, blood vessels, or the nervous system, and no lymph nodes have disseminated to distant organs [23]. The quantity of tumors is three or fewer, and the maximum diameter of an individual cancerous nodule is less than or equal to 3 cm. Barcelona Clinical Staging of Liver Cancer, BCLC stage 0 is the earliest stage of hepatocellular carcinoma, with tumors individually $\leq$2 cm; stage A is the early stage of hepatocellular carcinoma; stage B is the mid-stage of hepatocellular carcinoma development, stage C is the advanced stage of hepatocellular carcinoma and D is the terminal stage of hepatocellular carcinoma [24].

The most direct approach to early detection of liver cancer involves the implementation of molecular biomarkers. Despite the challenges of early-stage HCC detection, surgical resection, liver transplantation, and various chemotherapeutic interventions continue to be the primary treatment modalities, albeit with limited efficacy [25]. We have compiled a comprehensive summary of potential molecular markers for the early diagnosis of liver cancer.

At present, three main biomarkers - lens culinaris agglutinin-reactive fraction of AFP (AFP-L3), des-gamma carboxyprothrombin (DCP), and alpha-fetoprotein (AFP), are frequently utilized in clinical practice for the early detection of HCC [26]. AFP is frequently employed as a biomarker in various diagnostic approaches for identifying HCC. AFP is categorized as a glycoprotein and is distinguished by its glycan chain. Historically, AFP has been widely recognized as the standard for detecting liver cancer in clinical contexts. Nevertheless, the diagnostic precision for AFP-negative hepatocellular carcinoma (ANHC) is constrained due to the test's low sensitivity and specificity. This limitation primarily arises from the occurrence of ANHC cases, which frequently involve early-stage hepatocellular carcinoma lacking typical imaging attributes and not consistently producing AFP. Notably, elevated AFP levels are frequently observed in instances of cirrhosis or hepatitis [27]. However, the utilization of AFP as a surveillance tool for liver cancer detection in cirrhotic patients, through prospective specimen collection and retrospective blinded evaluation over 6 months, has yielded a considerable number of false positive results [28]. As a result, the exclusive reliance on AFP is no longer sufficient to meet the demands of clinical precision medicine. Consequently, there is an imperative need to redirect research endeavors toward the investigation and advancement of innovative biomarkers capable of accurately identifying initial indications of hepatocellular carcinoma and providing targeted medical interventions for patients.

3.1. Sample source of biomarkers for early liver cancer

Cancer detection relies heavily on biomarkers obtained from diverse bodily fluids such as blood, cerebrospinal fluid, saliva, and

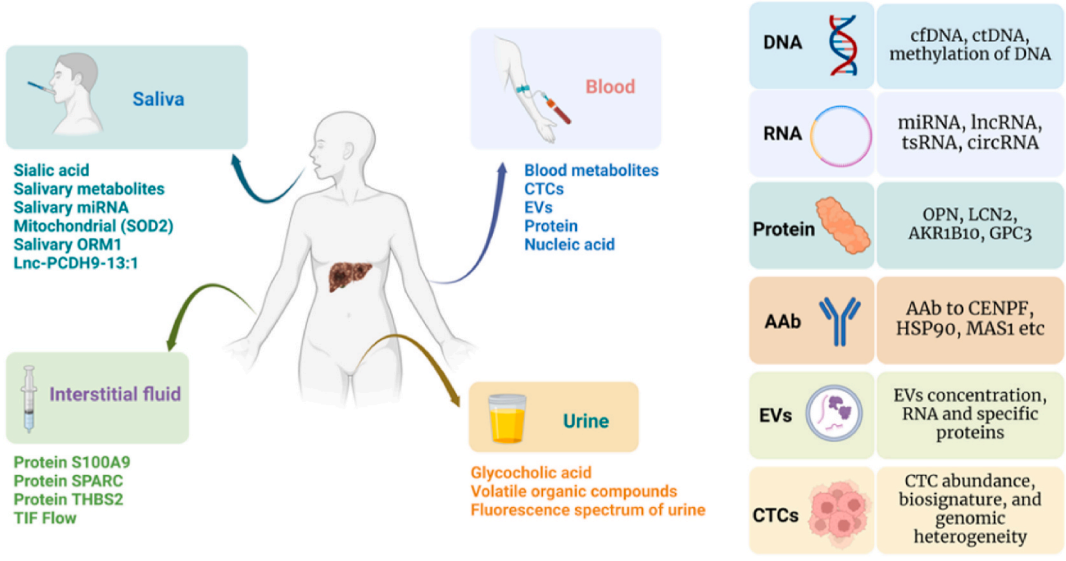

Fig. 1. Sample source of biomarkers for early liver cancer (image drawn with BioRender).

urine (Fig. 1). However, the quantity and attributes of these markers differ across fluid types. Consequently, the selection of sampling sites varies depending on the specific cancer type and marker under investigation. As medical standards advance, there is a growing inclination towards non-invasive testing methods and identifying novel biomarker sources. These sources enable detection using minute sample volumes.

Blood is commonly used for clinical marker testing, with serum being the preferred method for detecting early HCC markers [29]. There is a trend towards minimally invasive blood tests. Extracellular vesicles and exosomes contain RNA, DNA, and protein signaling molecules for intercellular communication [30]. Tumor interstitial fluid (TIF) can be used as a biomarker for early hepatocellular carcinoma, as its protein composition differs from serum and can be detected in peripheral blood [31–33].

3.2. Biomarkers for early diagnosis of liver cancer

Biomarkers are crucial for early detection of liver cancer and can improve precision medicine by indicating a patient's disease status. The biomedical community is focused on identifying and validating disease biomarkers, including those found in body fluids, for diagnosing liver cancer [34]. We summarized valuable biomolecules in body fluids as diagnostic liver cancer biomarkers.

3.2.1. Nucleic acid-based biomarkers

Advancements in sequencing technology have simplified the structure and composition of nucleic acids compared to proteins. Different types of RNA, including coding and non-coding RNA, play important roles in regulating gene expression. Non-coding RNA molecules like snoRNA, miRNA, siRNA, and piRNA are crucial in various cellular pathways. MiRNA and lncRNA are particularly important for cancer diagnosis [35,36]. The main way to detect HCC through DNA is by analyzing ctDNA and cfDNA for expression, integrity, mutation, and methylation [37]. This review focuses on DNA methylation and four non-coding RNAs for detection. Table 1 compares the detection methods and diagnostic performance of these markers.

Early liver cancer progression can be detected by DNA biomarkers like gene mutations and methylation events. However, recent studies show that gene mutations may lead to false positives as early diagnostic markers for cancer, while gene methylation is a more reliable marker for early-stage liver cancer. Early-stage HCC is associated with DNA hypomethylation of retrotransposons, filaments, proto-oncogenes, and methylation of key gene regulatory elements. Current DNA detection methods may require harsh chemical treatments that can degrade DNA and affect its stability [48]. Currently utilized DNA detection methods may require the application of harsh chemical interventions, leading to potential degradation of DNA and jeopardizing the integrity of the sampled genetic material. Multiple RNA sets within a cell provide a more comprehensive source of information than DNA. Changes in RNA expression levels often display more pronounced variations than those seen in proteins, with distinct RNA expression patterns being particularly notable during the transition from hepatitis or cirrhosis to hepatocellular carcinoma. Recently, LAMP has shown the ability to amplify 1–10 gene copies in 1 h. RT-LAMP and μLAMP are effective for detecting RNA markers. Although numerous biomarkers exist for HCC, only a limited number are clinically applicable, each possessing distinct advantages. Regardless of the type of markers used (DNA or RNA), it is important to study nucleic acid markers that provide better sensitivity and specificity for accurately diagnosing early-stage HCC.

3.2.2. Protein biomarkers

3.2.2.1. Protein. Protein biomarkers encompass a range of proteins, enzymes, hormones, and antibodies. In cancer patients, the proteome undergoes notable alterations in both type and quantity within the organism. In contrast to the genome, the proteome exhibits a high degree of dynamism and diversity, enabling it to transmit more information and respond to various biological signals, environmental factors, and external stimuli. These dynamic characteristics of the proteome facilitate significant changes, particularly in post-transcriptional mediated physiological processes, which may not be evident at the nucleic acid level. Consequently, proteins demonstrate heightened responsiveness in liver cancer patients, thereby offering potential benefits of precise medical treatment.

6.2.2.2. Autoantibody. Autoantibodies (AAb) attack the body's tissues and organs. High levels of AAb can cause harm, making their measurement important for diagnosing autoimmune diseases [49]. Tumors release tumor antigens to trigger an immune response in

Table 1
Comparison of nucleic acid biomarkers for early HCC detection.

Nucleic acid biomarkers		Detection Methods	Sample Source	AUROC	Sensitivity	Specificity	Ref
cfDNA		qPCR	Plasma	0.705	43.4 %	100 %	[38]
		Qubit dsDNA HS Assay Kit		0.820	62.5 %	93.6 %	[39]
ctDNA		WTB-PCR	Plasma	N.A.	43.0 %	100 %	[40]
		WGS	Plasma	0.874	69.9 %	95.0 %	[41]
Methylation of DNA		qPCR + BSP	Tumor tissue	0.957	92.0 %	98.0 %	[42]
tsRNA	tRF-Gln-TTG-006	qPCR	Serum	N.A.	79.0 %	74.8 %	[43]
circRNA	has-circ-0001445	qRT-PCR	Plasma	0.862	71.2 %	94.2 %	[44]
lncRNA	lncRNA-D16366	qRT-PCR	Serum	0.752	65.5 %	84.6 %	[45]
	LINC00853	RT-qPCR	Serum-EV	0.950–0.969	93.75 %	89.77 %	[46]
microRNA	miR-16	RT-PCR	Serum	0.798	91.0 %	58.0 %	[47]
	miR-122			0.759	83.0 %	64.0 %	

Table 2
Comparison of autoantibody biomarkers for early HCC detection.

Autoantibody	Detection Methods	Sample Source	AUROC	Sensitivity	Specificity	Ref
CENPF aAb	Protein microarray analysis	Serum	0.828	73.6 %	73.7 %	[50]
HSP60 aAb			0.779	79.3 %	62.2 %	
7-AAb panel	Protein microarray analysis	Serum	0.910	72.6 %	90.0 %	[51]
SF3B1 aAb	B cell hybridoma screen	Serum	0.873	73.5 %	91.8 %	[52]

patients, making TAA serum autoantibodies potentially useful for early detection of HCC in oncology. Table 2 compares detection methods and diagnostic performance of identified autoantibody markers.

6.2.2.3. Plasma protein panel. According to the AASLD, the diagnostic efficacy of a single biomarker in identifying early-stage HCC is significantly lower compared to a combination of multiple biomarkers. Consequently, a plasma protein panel comprising several protein biomarkers has the potential to provide more comprehensive diagnostic insights into the dynamic progression of HCC. The protein panel can be combined with several other early liver cancer protein markers, such as epithelial cell adhesion molecule (EpCAM), cell surface vimentin (CSV), transforming growth factor beta/sirtuin (TGF-β/sirtuin), and members of the DNA repair pathway, within novel protein panels facilitates enhanced early HCC detection efficiency [53].

Protein-based biomarkers exhibit greater detectability and species diversity than nucleic acid-based markers in the early detection of liver cancer. Despite the limited diagnostic performance of AFP, it remains the cornerstone of clinical diagnosis for early-stage HCC. Consequently, the current trend involves integrating novel protein markers with AFP to augment diagnostic efficacy and achieve precise medical interventions for patients. Table 3 summarizes the comparison of the detection methods and diagnostic performance of the recently identified protein markers mentioned. Investigating and enhancing the function of new protein markers are crucial due to their diagnostic significance in early HCC prediction. They possess potential not just as specific targets for HCC immunotherapy but also as prognostic indicators in personalized medicine for early-stage HCC patients.

3.2.3. Circulating tumor cell and extracellular vesicles

Circulating tumor cells (CTCs) encompass diverse subtypes of neoplastic cells. They detach from primary malignant tumor lesions, infiltrate the bloodstream, and manifest in the peripheral blood as either individual cells or clusters of cells [66]. CTCs found in the peripheral circulatory system are present in small amounts. However, their migratory capacity is noteworthy due to their ability to hinder cell adhesion through epithelial-mesenchymal transition (EMT). This transition enhances the cells' capability to de-differentiate and metastasize, facilitating their migration from the vasculature to the extravascular region. Some CTCs undergo a mesenchymal-epithelial transition (MET), acquiring intercellular adhesion capacity, evading capture, and adhering to tissues, ultimately leading to the development of metastases [67,68]. CTCs serve as a significant biomarker in the timely identification of HCC due to their pivotal involvement in metastasis initiation. Notably, early metastasis is often associated with phenotypes such as EMT and the formation of CTC clusters. By employing a single-cell analysis-based methodology, the comprehensive characterization of early tumor progression and the identification of molecular attributes can be achieved through the detection and analysis of CTCs in peripheral blood. This approach facilitates the selection of personalized medical interventions for individual patients, thereby enabling the realization of precision medicine during the initial stages of tumor development [69]. CTCs have the potential to be cultivated in a manner that replicates the physiological conditions of hepatocellular carcinoma, thereby creating a three-dimensional culture system known as an organoid. This organoid serves as a valuable tool for conducting early-stage studies on HCC [70]. The practice of liquid biopsy for liver cancer has gained popularity as a means of early detection for hepatocellular carcinoma. By continuously acquiring samples at various stages of liver cancer growth, it becomes possible to predict the onset of the disease. This, in turn, has spurred the investigation and detection of CTCs. The identification of early hepatocellular carcinoma can be achieved through the straightforward peripheral detection of CTC abundance, biosignature, and genomic heterogeneity [71]. In their study, Zhao et al. [72] analyzed the

Table 3
Comparison of protein biomarkers for early HCC detection.

Protein biomarkers/protein panel	Detection Methods	Sample Source	AUROC	Sensitivity	Specificity	Ref
Hsp90α	ELISA	Plasma	0.963	91.4 %	91.3 %	[54]
OPN	ELISA	Serum	0.851	79.2 %	79.6 %	[55]
LCN-2	ELISA	Serum	0.945	92.9 %	93.8 %	[56]
GPC3	ELISA; Flow cytometry	Serum	N.A.	85.0 %	95.0 %	[57]
AKR1B10	Time-Resolved Fluorescence (TRF)	Serum	0.896	64.6 %	92.3 %	[58]
DKK1	ELISA;	Serum	0.865	70.9 %	90.5 %	[59]
LMNB1	MALDI-TOF/TOF	Plasma	N.A.	76 %	82 %	[60]
VEGF	ELISA	Plasma	0.980	98 %	46 %	[61]
TGF-β	ELISA	Plasma	0.980	100 %	94 %	[62]
PTX 3	Quantikine Human Pentraxin 3/TSG-14 Immunoassay	Serum	0.956	90.6 %	91.2 %	[63]
P5	OPN, GDF15, NSE, TRAP5, OPG	Plasma	0.907	96.1 %	85.2 %	[64]
HepaClear	AFP + DCP+3 hypermethylated CpG sites	Plasma	0.848	68.4 %	96.2 %	[65]

proportion of epithelial CTCs (E-CTCs) and mesenchymal CTCs (M-CTCs) relative to the total CTCs count in patients diagnosed with HCC. The findings revealed a positive correlation between the total CTCs count and the prevalence of M-CTCs, indicating a higher degree of tumor malignancy and an increased likelihood of recurrence. The study also demonstrated acceptable levels of sensitivity and specificity, thereby suggesting the potential utility of CTCs as a biomarker for early detection of HCC.

EVs have been found to have various effects on liver cancer, including reducing immune response, remodeling the tumor microenvironment, and enhancing cancer cell proliferation, migration, and invasion. These alterations enable EVs to modulate immune tolerance, supporting liver cancer cell survival and promoting resistance to drug progression in liver cancer [73]. The transmission of many RNAs (ucRNA, lncRNA, and circRNA), enzymes (glycolytic enzymes, metalloenzymes), and proteins (14-3-3ζ) via EVs between hepatocellular carcinoma cells has been observed to promote hepatocellular progression and metastasis, creating a favorable environment for hepatocellular carcinoma survival and aiding in the evasion of immune surveillance. Hence, the significance of EVs in the early detection of HCC has been emphasized [74]. The identification of early-stage HCC can be accomplished through the assessment of extracellular vesicle concentration, the presence of extracellular vesicles expressing altered non-coding RNAs, and specific proteins [75]. In a study conducted by Henrike et al. [76], the utilization of fluorescence-activated cell scanning enabled the detection of large extracellular vesicles, specifically annexinV^+ $EpCAM^+$ $CD147^+$ taMPs, which effectively differentiated HCC patients from those with cirrhosis and without tumors (with a sensitivity and specificity exceeding 78 %).

CTCs and EVs provide more detailed real-time monitoring than tumor markers, capturing changes at epigenetic and protein levels [77]. They offer insights into tumor mutation burden and patient mutation profiles, with PD-L1+ CTCs and SF3B4+ EVs being able to distinguish early-stage HCC patients accurately [78,79].

In addition to the CTCs described above, the heterogeneous cancer cell populations of HCC: cancer stem cells (CSCs), TEM, cancer-associated fibroblasts (CAFs), and hepatic stellate cells (HSCs) have potential as markers for the diagnosis of early-stage liver cancer [80]. This contrasts with ctDNA, which is limited to identifying mutations, deletions, and methylation fragmentation. CTCs offer a more extensive range of diagnostic insights for individuals with early-stage liver cancer due to their enriched complete genomes, transcriptomes, proteomes, and other factors [81]. Nevertheless, most clinical techniques employed to detect CTCs necessitate intricate processes for concentration and enrichment, as CTCs typically exist in peripheral blood at minimal concentrations amidst a high background of hematopoiesis. If the CTCs test is implemented in practical clinical settings, the limited blood volume obtained from a single patient is insufficient for conducting the CTCs test. However, EVs exhibit enhanced content stability compared to CTCs due to the protective lipid bilayer membranes, resulting in improved targeting capabilities and resistance to matrix interference. However, similar to CTCs, EVs encounter challenges in clinical testing, such as the presence of complex proteins and lipids that can hinder the purification and enrichment processes [82]. In subsequent developments, the amalgamation of microfluidics, SERS technology, and nanomaterials holds substantial potential for enhancing the enrichment of CTCs and EVs. This advancement could facilitate accurate diagnosis of individuals afflicted with early-stage liver cancer.

3.2.4. Serum metabolite biomarkers

Blood metabolites contain numerous biomarkers and carcinogens, with some being endogenously produced and others being acquired from the environment. In terms of selecting between plasma and serum metabolites, serum metabolites exhibit exceptional diagnostic characteristics. Because of their lack of fibrinogen, simpler composition compared to plasma, a diverse array of endogenous small-molecule metabolites, and their significance as essential samples for metabolomics analysis of bodily fluids [83]. The histological analysis of serum metabolite variables has proven to be a highly sensitive method for the early detection of liver cancer changes, as even minor alterations in metabolites can be detected. Analytical instruments such as mass spectrometry (MS) and ultra-high-performance liquid chromatography (UPLC) are commonly employed for the analysis of serum metabolites in early HCC. Notable metabolite markers that have been identified include 1-methyladenosine, glucodeoxycholic acid, and taurocholic acid [84]. Han et al. [85] employed high-performance liquid chromatography (HPLC) to conduct metabolomics analysis on pairs of HCC tissues and distal non-cancerous tissues in HCC patients. Their findings revealed a significant correlation between the presence of retinol metabolites (such as retinol, retinol, retinoic acid, etc.) in HCC tissues and the growth and differentiation of hepatocellular carcinoma. Notably, the levels of these metabolites were observed to be lower in both HCC tissue and serum compared to cirrhotic and healthy populations. In terms of differentiating early HCC from cirrhosis, retinol and retinal exhibited superior performance when compared to AFP, with a sensitivity of 80 % versus 48.8 %. Lu et al. [86] employed mass spectrometry to analyze the hepatic metabolism of patients with HCC. Their findings revealed a significant correlation between the serum metabolite acetylcarnitine and differentiated HCC. Moreover, the levels of acetylcarnitine exhibited a decreasing trend as the HCC progressed from stage T1 to T4. Notably, the diagnostic accuracy of acetylcarnitine in distinguishing HCC patients from those with cirrhosis and the healthy population was determined to be 74 % sensitivity and 79 % specificity.

In the context of early diagnosis of HCC, the utilization of a panel consisting of serum metabolic biomarkers has been explored. Pin et al. [87] conducted a study wherein they developed a panel comprising eight serum metabolite biomarkers, namely phenylphenyl-tryptophan, ethanoic acid, and taurobenzoic acid, through the application of HPLC. The sensitivity of this diagnostic test in differentiating individuals with liver cancer from those without liver cancer prior to clinical diagnosis was found to be 80%–70.3 %, surpassing the diagnostic performance of AFP. The panel consisting of glycine, aspartic acid, SM (42:3), and SM (43:2) demonstrated a sensitivity of 75 % and a specificity of 90 % in the diagnosis of primary sclerosing cholangitis (PSC) and HCC [88].

A significant portion of early liver cancers arise from non-alcoholic steatohepatitis (NASH) resulting from irregular dietary patterns. Fat accumulation due to suboptimal eating habits can disrupt serum metabolites and gastrointestinal flora. Additionally, the gastrointestinal biome serves as a predictive factor for serum metabolite levels, and the relationship between the two is mutually reinforcing [89]. Consequently, minor alterations in gene and protein expression have a magnified impact on metabolites, facilitating

their detection. The identification of endogenous small molecules in the serum metabolites of individuals potentially diagnosed with early-stage hepatocellular carcinoma is achieved through a non-invasive assay technique. The technique also systematically captures a broad spectrum of information on human homeostasis and serum [90]. Nevertheless, the concentration range of serum metabolites varies significantly, and current detection methods, such as GC-MS and LC-MS, cannot simultaneously and comprehensively analyze multiple indicators. Furthermore, the existing spectral libraries and databases of serum metabolites remain incomplete.

3.2.5. Gut microbiota biomarkers

The liver, being the initial organ to encounter microbial products in circulation and affected by the intestinal microbiota, engages in interactions with components or metabolites of the intestinal microbiota through diverse mechanisms [91]. Its primary role in regulating microbial activity is primarily accomplished through the secretion of bile acids. The occurrence of intestinal leakage is a catalyst for the progression of HCC via the MAMP-TLR pathway, and it represents a significant characteristic of chronic liver disease. Bacterial dysbiosis exacerbates the infiltration of hepatocellular carcinoma cells by suppressing the immune response of the host. Simultaneously, microbe-associated molecular patterns exacerbate hepatic tissue degeneration, and promote hepatic fibrosis, consequently influencing the early progression of hepatocellular carcinoma [92,93].

Early HCC is closely linked to the presence of Escherichia coli in the gastrointestinal tract. The occurrence of a greater number of microbial species is associated with a reduction in butyric acid-producing genera and an increase in the abundance of lipopolysaccharide-producing bacteria with pathogenic properties. In HCC cases associated with non-alcoholic fatty liver disease, there is an increase in the prevalence of Anaplasma and Ruminalococcaceae, while Bifidobacteria are found to be diminished [94]. These alterations create an oncogenic microenvironment that indirectly induces DNA damage, disrupts the secretion of hepatic stellate cells, and up-regulates the expression of cyclooxygenase-2 (COX-2), thereby promoting the development of HCC [95]. Ren et al. [96] conducted a study where they collected fecal samples from individuals diagnosed with HCC, cirrhosis and healthy individuals. They utilized Miseq sequencing to analyze the gut microbial composition. The results revealed that in the early stages of liver cancer, actinomycetes increased, butyrate-producing genera decreased, and lipopolysaccharide-producing genera increased compared to the control group with cirrhosis. To validate these findings, a panel of 30 microbiological markers was selected. The study found that the POD index achieved an area under the curve (AUC) value of 80.64 % when distinguishing between early HCC and non-HCC cohorts, with a 95 % confidence interval (CI) ranging from 74.47 % to 86.8 %.

The rational regulation of gut microbes within the microbe-gut-liver axis has the potential to be utilized as a therapeutic approach for the treatment and prevention of HCC [95]. In obese individuals, the consumption of high-fat diets and the presence of lipopolysaccharides and deoxycholic acid can result in intestinal permeability and subsequent hepatocellular damage. This process compromises the body's immune system and contributes to the progression of chronic liver disease towards hepatic fibrosis, cirrhosis, and HCC. Furthermore, the non-invasive and efficient utilization of gut microbes as diagnostic markers holds promise for the early detection of liver cancer. Although many experiments have demonstrated that gut microbes are associated with early-stage HCC, further research is needed for clinical application.

In Table 4, we have provided a summary of the benefits and drawbacks associated with the utilization of these markers for the early detection of liver cancer. Despite the advancements in proteomic analysis methodologies that have contributed to the identification of protein-based biomarkers, it is important to note that no singular protein marker has yet exhibited impeccable sensitivity and

Table 4
Classification and comparison of biomarkers for early-stage HCC.

Biomarker type	Categorization	Dominance	Drawbacks
Nucleic acid	RNA	1 High sensitivity and specificity 2 Dynamic responses to cellular states and regulatory processes 3 The variability of expression is typically greater compared to the protein level	1 Insufficient criteria for detection methodologies. 2 The volume of sequencing data is substantial, and the sequencing technique and procedure impact the data's quality 3 Limited comprehensive and extensive clinical validation. 4 Elaborate mechanisms of action within tumor biology
	DNA	1 The detection process is straightforward 2 Established methods of separation are available 3 The technique offers a diverse range of genetic information and a comprehensive assessment of tumor mutation data	1 Not suitable for therapeutic monitoring, pharmacodynamics assessment 2 Low mutation frequency and technical challenges in accurately discerning tumor variants from background noise
Protein	Enzymes, Hormones, Antibodies	1 The facilitated accessibility of samples 2 High degree of dynamism, effectively responding to and adapting to biological signals and environmental factors	1 Poor stability and susceptibility 2 Most instruments for detecting proteins are not very sensitive
CTCs	cell	1 Wide range of analytes for analysis 2 Tumor source specificity 3 capability to conduct in vitro experiments	1 Low cell number 2 Enrichment methods can have an impact on the detection of heterogeneity in CTCs
EVs	Exosome, apoptotic body, microve sicles, migrasome	1 Rich in DNA, RNA, proteins and metabolites 2 Ability to perform in vitro tests 3 Stable, maintaining the original source of cellular biological information	1 Higher technical difficulties in extraction and purification

specificity in diagnosing individuals with early HCC. Further investigation and validation of sample handling and potential interference from other products in clinical practice are necessary to fully understand the diagnostic potential of serum metabolites and gastrointestinal flora in early-stage HCC patients. The combination of multiple proteins for testing, along with co-testing with nucleic acid classes, can enhance the efficiency of patient testing. Moreover, individualized risk stratification and monitoring of patients with diverse biomarker diagnostics, considering factors such as physical conditions, disease sites, and cancer causes, are crucial for effective patient management.

4. POCT technology and platform for early liver cancer detection

The current emphasis in precision medicine research is on the advancement of biomarkers. The timely identification of suitable liver cancer markers during the early stages of liver cancer development holds immense importance for personalized testing and medical intervention. However, the identification of novel biomarkers necessitates the use of highly accurate detection instrumentation, a gap that is effectively filled by the rapid progress of POCT. The introduction of POCT devices has facilitated the prompt diagnosis of early HCC regardless of temporal and geographical constraints. The development of a POCT device specifically designed for early HCC detection holds the potential to enhance patient identification and survival rates while also enabling personalized medical interventions. The subsequent section outlines various established POCT technologies and platforms utilized for the early detection of HCC.

4.1. POCT technology

4.1.1. Colorimetric

Colorimetric sensors offer a promising POCT technology for early liver cancer detection at home. Patients can assess their condition qualitatively through color changes, which can then be converted into numerical values using a smartphone for personalized diagnosis.

To enhance the colorimetric technology utilized in the POCT device, including a smartphone can facilitate the interpretation of colorimetric outcomes. Lewińska and her colleagues [97] replaced the conventional Jaffé colorimetric detection method with 3,5-dinitrobenzoic acid (DNBA). They employed a modified smartphone created through 3D printing, along with a specialized computer vision algorithmic application, to automatically identify and interpret the colored regions for signal detection. Signal amplification strategies for smartphones lack selectivity. Another prevalent trend involves utilizing the distinctive optical properties of gold nanoparticles or gold-standardized silver stains, along with biomolecular enzymatic reactions, as detection probes. The utilization of such detection probes significantly enhances the sensitivity and selectivity of the target substance being measured, enabling direct observation of the results with the naked eye, even in the presence of a high concentration of the target molecule. Gong et al. [98] cleverly combine 3D printing with the colorimetric reporter AuNPs. Upon detection of AFP in a clinical patient's blood, the AFP is introduced into the multichannel for an enzyme immunoassay, which facilitates the synthesis of ascorbic acid that aggregates the AuNPs, resulting in a colorimetric change. However, the authors initially made a basic diagnosis using naked-eye observations, then confirmed it with a precise diagnosis using multichannel photocurrent changes. Analyzing color changes from AuNPs with a smartphone can lead to high selectivity and sensitivity. Wang et al. [99] fabricated a hybrid hydrogel film consisting of gold nanoparticles and DNA as a signaling component capable of releasing AuNP as a colorimetric signal output upon the identification of a specific liver cancer biomarker, miRNA-21. Subsequently, an enzyme-free entropy-driven dynamic DNA network (EDN) was used as a signal converter and amplification unit, allowing accurate quantitative colorimetric results to be obtained by intelligent image analysis techniques using only mobile phones.

However, recent research has demonstrated that the distance dependence of AuNP poses challenges in monitoring color changes when the concentration of the target substance is insufficiently low. Consequently, Lee and colleagues [100] employed graphene oxide (GO) peroxidase mimicking DNAzyme (Dz) to facilitate the detection of the liver cancer biomarker miR-122 with a highly sensitive sensor. After targeted recognition of miRNAs, Dz captured and collected by GO can assist in enhanced colorimetric development, resulting in the rapid display of very low miRNA concentrations on paper within a few seconds. In a similar vein, Wang et al. [101] have devised a dual aptamer target-binding colorimetric assay devoid of enzymes, which effectively combines the exceptional specificity of vanadium aggregates with the catalytic prowess of the targets. Supported by bimetallic PtAu nanoparticles, which have superior chromogenic performance to peroxidase, cancer cell detection in serum samples from hepatocellular carcinoma patients can be better achieved. Consequently, this assay exhibits remarkable sensitivity and specificity in the identification of hepatocellular carcinoma cells, with a limit of detection (LOD) of 10 cells/ml.

Visual colorimetry is dependent solely on human observation of color outcomes, resulting in significant subjective errors. Consequently, it is exclusively employed for qualitative and semi-quantitative analyses. Conversely, photoelectric colorimetry exhibits enhanced accuracy and is well-suited for the quantitative and precise identification of biomarkers within living organisms. However, due to the exorbitant expenses and intricate nature of the equipment, as well as the utilization of photoelectric colorimetry involving a photocell, tungsten light source, and filter as a measurement tool, this method is primarily applicable for meticulous analysis and research conducted within laboratory or specialized environments. In contemporary times, POCT devices leverage ImageJ software integrated into smartphones, enhanced with 3D printed components, to identify the desired region and compute the average RGB value after capturing an image of the resultant colorimetric shot. This innovative approach ultimately facilitates the quantitative assessment of the targeted biomarkers. To enhance the capabilities of colorimetric analysis techniques, it is imperative to develop novel signal amplification platforms or methods that can be seamlessly integrated with intelligent programs and mobile phone technologies in the future.

4.1.2. Electrochemical technology

Electrochemical sensors need more complex components and procedures than colorimetric sensors, which allow for more innovation in identifying early liver cancer biomarkers. While primarily used for experimental purposes, advancements in materials and methods can lower development costs and increase usage.

HCC biomarkers exhibit low expression during the initial phases, which has led many researchers to investigate the potential of electrochemical techniques for the precise detection of early-stage cancers. These techniques are renowned for their sensitivity, speed, high resolution, and reproducibility. Several electrochemical biosensors, including flexible and homogeneous biosensors, have been developed to aid in accurate diagnosis [102,103].

Researchers are increasingly employing novel nanomaterials such as carbon nanoparticles, nanochannels, and graphene to enhance the sensitivity of electrochemical detection by modifying electrodes [104]. In a study conducted by Zhang et al. [105], Au nanoflowers (AuNFs) combined with peptide nucleic acids (PNA) were utilized to modify carbon fiber microelectrodes (CFME). The resulting PNA probe on AuNFs-CFME exhibited remarkable stability and resistance to interference, rendering it suitable as a working electrode (WE) for ultrasensitive assays. Meanwhile, the multiple active sites provided by AuNF allowed the detection of the hepatocellular carcinoma marker circCDYL in serum at very low levels (3.29 fM) right from the smartphone. Furthermore, highly sensitive electrochemical immunobiochips utilizing porous three-dimensional graphene aerogel (3D-GA) were employed to detect different tumor markers in liquid samples through the application of electrochemical impedance spectroscopy (EIS). Using this device, the authors' team achieved a detection range of 1.0×10^{-8}-1.0×10^{-5} and a LOD of 7.9 pg/mL in just 5 μL of AFP sample, which greatly improves the efficiency of the detection of early-stage hepatocellular carcinoma markers [106].

Electrochemical analysis techniques utilized in POCT are seamlessly incorporated with microfluidic chips, paper-based sensors, and single-stranded nucleic acid chain (aptamer) point-of-care (POC) devices, thereby facilitating the analysis of early liver cancer markers through multiple sensor arrays. In certain less developed regions, self-powered paper-based POC devices and flexible wearable energy supply devices are also deemed indispensable.

4.1.3. Fluorescence technology

Fluorescence technology is used for in vitro POCT detection of early liver cancer markers, improving accuracy, sensitivity, and specificity through new fluorescent reagents.

Fluorescence detection technology can effectively analyze and identify proteomics and genomics for the early diagnosis of HCC, facilitating the sensitive detection of associated biomarkers. This capability facilitates the provision of accurate medical interventions for patients. Fluorescence imaging in the near-infrared second region is widely used in in vivo local imaging of tumors and in vivo internal imaging of living tissues, such as microangiography, due to its high imaging resolution and depth of penetration [107]. Various fluorescence detection methods are available, including fluorescence spectroscopy, photochemiluminescence, and fluorescent probe techniques. In the context of detecting antibodies for early HCC biomarkers, the utilization of fluorescently labeled antibody detection techniques presents notable benefits. These techniques enable the characterization of antibody expression through the incorporation of fluorescein isothiocyanate, rhodamine fluorescein, and other similar compounds. Fluorescence detection systems utilized in POCT generally comprise a light-emitting diode (LED) responsible for generating excitation light alongside a photodiode that detects fluorescence and generates a current proportional to the fluorescence intensity [108]. Due to the relatively weak current generated by the photodiode, the converter's processing becomes necessary to amplify or convert the current signal, achieving the heightened sensitivity requisite for the fluorescence detector. Ryu et al. [109] demonstrated that the utilization of Lee Filters' short-pass and long-pass filters, along with a green LED as an excitation light source, enhances the signal-to-noise ratio. It also facilitates the implementation of a reflective polarisation film (DBEF) in liquid crystal displays, thereby augmenting brightness.

The enhancement of biomolecular fluorescent sensors can be achieved through the development of fluorophores with increased quantum yields, improved tissue uptake, and the modification of substrate substrates. Cheng et al. [110] investigated the utilization of an enzyme-free catalytic hairpin assembly (CHA) technique to detect AFP and GPC3, early indicators of HCC. The generation of N-methyl mesoporphyrin IX (NMM) fluorescence and DQ bursts are controlled by amplifying single-stranded DNA and initiating reactions through CHA after target protein recognition, enabling simultaneous detection of AFP and GPC3. Similarly, Li et al. [111] developed an integrated sensor known as a homogeneous magnetofluorescent exosome (hMFEX) nanosensor. The process of immunomagnetic capture of tumor exosomes serves as a catalyst for the assembly of DNA three-way junctions in homogeneous solutions with a polymerization-induced luminescent agent and graphene oxide, resulting in the amplification of fluorescence signals. The utilization of innovative nanomaterials, such as ZnO nanorod structures, in the construction of fluorescent biosensor substrates has the potential to improve the fabrication of a fluorescence enhancement substrate. Its direct assembly into a customized array format can effectively improve the signal-to-noise ratio of biomolecular analysis [112].

To enhance the signal intensity for the detection of liver cancer biomarkers at low concentrations, the incorporation of plasma metal nanomaterials, semiconductor nanocrystalline quantum dots, and novel fluorescent dyes can be considered. The integration of POCT-integrated fluorescence detection technology facilitates the sensitive and real-time visual detection of target compounds at low concentrations, employing appropriate fluorescent probes. Nevertheless, fluorescence detection techniques frequently necessitate calibration due to interferences arising from environmental factors, instrumentation, and light sources. Consequently, ratiometric detection continues to serve as the primary approach in fluorescence technology for the quantitative detection of early liver cancer markers.

4.1.4. Surface plasmon resonance (SPR)

SPR offers the benefit of facilitating label-free detection and continuous monitoring in real time. These sensors can detect changes

in the local refractive index, thereby enabling the investigation of various interactions, such as those involving protein-nucleic acid and antigen-antibody interactions [113]. SPR can directly interact with cancer-related fluid samples, including blood, saliva, and urine. It enables the detection of target biomolecules even at low concentrations with remarkable sensitivity. Consequently, SPR has gained significant popularity in POCT for the early detection of various types of cancers.

The utilization of SPR in the early detection of HCC by POCT devices primarily relies on antibodies as biorecognition receptors. Nevertheless, the affinity and bioactivity of these antibodies remain relatively low, necessitating the implementation of certain technologies or platforms to amplify the detection signal. To enhance detection efficiency, initial advancements have involved the integration of fiber optics with sensors, such as optical coupling to smartphones, modification of plastic fibre POFs, and signal enhancement with gold nanomaterials. Ucci's team [114] has successfully integrated a sandwich immunoassay into the Au surface plasmon resonance sensor. A sandwich immunorecognition configuration was employed to selectively identify and immobilize the target molecule on the Au surface, thereby enhancing the recorded signal. The efficacy of this approach was demonstrated by successfully validating the early liver cancer marker AFP at a detection level of 15 ng/ml. In contrast, Zeni [115] has developed a streamlined SPR-Polymer POF device that is both portable and compact, eliminating the need for a sandwich signal amplification strategy. This innovative device utilizes a photoresist layer and a thin gold nanoparticle deposition overlay, followed by applying a high surface density target antibody onto the POF sensor. Combining POF with nanoparticles has been found to generate localized surface plasmon resonance and enhance sensitivity. By modifying various early HCC target protein monoclonal antibodies on POF fibre optics, a rapid assay can be achieved that is superior to the complex procedure of ELISA. Nevertheless, sandwich immunoassays are not suitable for the analysis of nucleic acids. To address this limitation, Yeung's team [116] has developed a transport surface plasmon resonance (t-SPR) technique for miRNAs, eliminating the need for extended RNA samples. This technique involves the integration of a PMMA microfluidic chip with a functionalized capped gold nano-slit (CG nano-slit) sensor. The T-SPR technique utilizes a dual-hybrid methodology, wherein the target RNA forms a specific and complementary pairing with the monitoring probe, leading to binding with the sensor surface and generating asymmetric Fano resonance peaks. However, Customized t-SPR requires complex automated equipment and a microfluidic chip. Nucleic acid probe molecules cannot be covalently modified on metallographic substrates like antibodies. Li et al. [117] then used a biotin-affinity system as a medium to bind a DNA probe for miRNA-125b, a diagnostic miRNA for early HCC, to the surface of the chip to achieve the capture of target RNA molecules.

SPR technology can detect transparent and colored samples (blood, urine, saliva, etc.), and the turbidity of the material does not alter its sensing power. The development of future SPR sensors necessitates further investigation in terms of miniaturization and complete integration. In the future, the efficacy of early liver cancer marker detection could be enhanced, and personalized medicine for patients could be facilitated through the utilization of various advancements such as biofunctionalized nanoparticles, micrometallic materials, enzyme utilization, and the integration of multiple materials to modify SPR fiber-optic probes.

4.1.5. Surface enhanced Raman scattering (SERS)

SERS allows for efficient detection of early liver cancer biomarkers, but complex data requires intelligent algorithms for patient analysis. Portable SERS detectors have been created for use outside of traditional laboratory settings, making detection technology more accessible to patients.

SERS quantitative analyses frequently employ labeling techniques that yield robust signals exhibiting distinctive spectral attributes. The utilization of metallic nano substrates enables SERS to furnish an extensive vibrational spectrum, which is essential for ensuring life safety, conducting food testing, and facilitating medical testing [118]. SERS plays a crucial role in medical diagnosis applications owing to its extensive detection range and heightened sensitivity. A variety of SERS substrates have been developed to enhance the detection signal. Ma et al. [119] have made significant contributions in this area by introducing innovative approaches such as metal nanoparticles or metal nanoflowers, arrays of metal pores, improved molecular interaction, and the exploration of novel substrate materials, including semiconductors like graphene and quantum dots. In their study, Ma et al. have successfully identified a functionalized porous anodic aluminum oxide (AAO) gold nanoparticle substrate and AuMBA@Ag core-shell nanoparticles as effective SERS substrates. They divided the AAO substrate into two regions, each of which was modified with AuNPs and Au@Ag, respectively. Microfluidic actuation was then performed to achieve dual detection of the early HCC marker miRNA-21. Meanwhile, the multiplexing capability of SERS enables the simultaneous detection of multiple early HCC markers. However, non-labeled SERS assays often require complex peak spectra to be resolved one by one. There are two approaches here that achieve high selectivity while also multiplexing the detection of early HCC markers. The first is the creation of multiple differently encoded SERS NPs: recognition and detection by multiple Raman reporter molecules and monoclonal antibodies or probing DNA-modified gold and silver nanoparticles of various morphologies. Zhou et al. [120] then modified three miRNA markers (miRNA-21, miRNA-122, miRNA-223) capture probes for early HCC diagnosis on star-shaped gold nanoparticles. They meanwhile modified three Raman tags: R6G, crystalline violet (CV), and 4-amino thiophenol (4-ATP) to characterize the markers separately. The other is to chemically modify different Raman reporter molecules on gold and silver nano substrates and subsequently functionalize each miRNA probe/monoclonal antibody sequentially according to the reporter molecule with activated carboxylate coupling function. Zhao et al. [121] sequentially chemisorbed three Raman reporter molecules (MBA, DSNB, and 6 GT) in vertically oriented 3 μm wide line arrays of silver nanofilms using microcontact printing. This was followed by sequential DNA probe/AFP antibody modification on Raman label-functionalized substrates using EDC/NHS. Finally, the resulting SERS frequency offset was used to detect early HCC markers: miR-26a-5p, miR-233, and AFP.

The utilization of computer technology has led to the growing application of artificial intelligence in the field of medical analysis and testing. Deep learning (DL), an artificial intelligence methodology, is employed to automate intricate data classification and analysis. The integration of DL in label-free SERS detection of early HCC enables a more precise assessment of serum samples for the early detection of HCC disease [122]. Wang et al. [123] fused DL based on innovative novel silicon-based bimetallic

(SiTITPs@Ag@AuNPs) ultrasensitive nano substrates, which ultimately achieved 98.75 % predictive accuracy in cirrhosis, hepatitis B, HCC, and healthy populations. In the field of single-molecule detection, the intensity and spectral profile of Raman spectra exhibit notable variations among individual molecules, rendering linear-based methods inadequate for their detection. However, the utilization of deep learning techniques enables the capture and categorization of information within these ranges [124].

The utilization of SERS technology facilitates the examination of minute quantities of diverse liver cancer biomarkers early due to the advantages of the high sensitivity. However, the lack of specificity in detection is attributed to the non-specific adsorption of analyte molecules to the sensor and the occurrence of structural changes during placement. Moreover, the heavy linearity and stability of the substrate for SERS spectroscopy are difficult to control. There is a need to continue to develop substrates with high stability. One of the ultimate goals for developing SERS substrates is obtaining high stability while maintaining its sensitivity.

4.1.6. Bioluminescence

Bioluminescence technology is a simple and effective way to diagnose liver cancer in low-resource areas. Maintaining the bioactivity of its reagents is crucial for successful use, requiring strict control during design, production, and transport. Developing new bioluminescent luciferins and luciferase can enhance its capabilities.

Variations in luciferin-luciferase systems and the expression of luciferase lead to the emission of light at different wavelengths. In vivo, the utilization of bioluminescence imaging (BLI) allows for the longitudinal assessment of early HCC through non-invasive and real-time analysis at the biomolecular level. Genetic engineering employs the stable expression of fluorescent enzymes or fluorescent proteins as biomarkers, such as the green fluorescent protein (GFP) derived from jellyfish. This technique is designed to facilitate the detection of various cancer-related processes, including cell genesis, metastasis, metabolism, apoptosis, and hypoxia through the expression of luciferase or proteins [125]. Consequently, it has gained extensive utilization in the fields of genetics, bioengineering, and cell biology. Additionally, BLI enables the identification of protein interactions, in vivo tracking of target cells, and monitoring of specific genes [126].

There has been a notable increase in the utilization of POCT bioluminescent sensors for the early detection of HCC, primarily

Table 5
Comparison of electrochemical, optical, fluorescent, and Sers biosensors.

Detection Methods	Principle	Detection Signal	Advantage	Defect
Electrochemical Biosensor	Electrochemical reactions (converting information about target molecules into electrical signals)	Current, Voltage	1 High specificity 2 Low background noise 3 High signal-to-noise ratio 4 Reusable 5 Rapid detection	1 Low LOD 2 Electrochemical matrix effect 3 Poor stability of biological components and difficulty in preparing biosensor component 4 Poor anti-interference capability
Fluorescent Biosensors	Fluorescent labelling (detection of fluorescence intensity and fluorescence lifetime)	Fluorescent signal	1 High selectivity 2 Real-time detection 3 High throughput 4 Quantitative analysis	1 Short lifetime and poor stability of fluorescent dyes 2 Vulnerability to environmental factors 3 Intrinsic fluorescent properties of tissues or cells generate non-specific background signals 4 Fluorescent labels have the potential to interact with the target molecule and thus produce false results
SERS Biosensors	Changes in refractive index due to the interaction of target molecules and plasmonic resonance waves on metal surfaces	Raman spectroscopy	1 High sensitivity 2 Multi-detection capability 3 Fingerprint recognition capability	1 Complex samples may produce interfering background signals 2 Limited in high-speed analysis due to its relatively long acquisition time 3 Poor reproducibility
Colorimetric Biosensors	Colour rendering reactions to produce colored compounds	Colour depth	1 Qualitative analyses with the naked eye 2 Economic accessibility	1 Limited absorbance range 2 Requirements for light source stability and wavelength selection
SPR Biosensors	Light resonates with plasma waves	Resonance angle	1 No labelling 2 High-throughput 3 Small amount of sample is required 4 Fast	1 Low specificity 2 Poor detection limits 3 Need for large and complex equipment
Bioluminescence Biosensors	Light signals generated by biochemical reactions of proteases produced by luciferase gene-tagged cells with corresponding substrates	Altered fluorescent signals	1 High signal-to-noise ratio 2 No external light source required 3 Suitable for deep tissue imaging	1 Weak fluorescence intensity 2 Unstable during the detection process

employing the bioluminescence tomography (BLT) method, which is calibrated by bioluminescence intensity decay. This method has been specifically employed for the in-situ detection of early HCC. However, the current bioluminescence sensor signal for early HCC detection remains relatively weak. To address this limitation, Chen et al. [127] conducted a study on a dual enzyme-mediated bioluminescence sensor that utilizes alkaline phosphatase (ALP) to degrade ATP into adenosine monophosphate (AMP), thereby inhibiting the luciferin-luciferin-ATP bioluminescence reaction. The luciferase-catalyzed reaction exhibits a heightened sensitivity to ATP, resulting in a decrease in bioluminescence intensity. This enhanced sensitivity enables the detection of early HCC using an ATP detector, which measures luminescence intensity. By incorporating bioluminescence resonance energy transfer (BRET) into fluorescent probes composed of HaloTag-fused NanoLuc luciferase (H-Nluc) and small-molecule probes modified with a chloroalkyl linker (H-TMR-HA). The emission of bioluminescence from luciferase can stimulate the receptor to generate a ratiometric signal, producing a discernible change in luminescent color within the BRET signal [128]. Xie et al. [129] screened the phage Affimer binding peptide library for Affimer proteins capable of specifically binding to GPC3, a marker for early hepatocellular carcinoma. They used sandwich chemiluminescence immunoassay to synthesize a complex of Affimer and the monoclonal antibody Mab and relied on the luminescence intensity generated to detect GPC3. BRET is mostly applied in antibody detection, utilizing the typical Y structure of antibodies. In contrast, most of the early liver cancer markers are irregular proteins, which requires innovation in recognizing peptide sites of BRET sensor proteins. Protein recognition sites compatible with BRET assays can usually specifically recognize target proteins using Affimer, Affibody, Alphabody, etc.

Bioluminescence technology, known for its low limit of detection (LOD), high signal-to-noise ratio, and exceptional selectivity, finds extensive application in identifying antibodies against endemic pathogens and in vivo imaging. A mobile application (APP) equipped with integrated smartphone capabilities and customized algorithms effectively captures bioluminescent signals, providing prompt feedback to the patient. Additionally, colorimetric analysis is employed to mitigate errors arising from the decline in absolute intensity caused by the depletion of the fluorescein substrate. Notably, the compact size (19.1 kDa) of the novel luciferase NanoLuc does not compromise its luminous output, enabling rapid detection of antibodies. However, the enzymatic activity of NanoLuc exhibits a decrease when employed in POCT for the identification of blood biomarkers indicative of early liver cancer. The comprehensive commercialization of bioluminescence technology for integration into POCT devices necessitates consideration of substrate and reagent expenses, as well as the stability of luciferase and fluorescent proteins. Herein, we compare the main advantages and disadvantages of electrochemical, optical, fluorescent, and SERS biosensors (Table 5).

4.2. POCT platform

4.2.1. Microfluidics

The microfluidic platform can detect liver cancer biomarkers and capture CTCs, making it crucial for personalized early-stage diagnosis. Novel nanomaterials and analytical platforms have advanced the development of these platforms. Microfluidics technology is widely used in biomedical analyses due to its cost-effectiveness, speed of analysis, and capacity to work with tiny amounts of chemicals and materials. Microfluidic models offer an alternative to conventional Petri dish assays by enabling the simulation of intricate tumor microenvironments. They encompass diverse cell and tissue cultures, such as 2D and 3D cell cultures, as well as tissue organoid cultures. Furthermore, the utilization of 3D bioprinting and decellularized scaffolds allows for the replication of tissue complexity necessary for certain types of cancers, thereby facilitating the provision of predictive responses for precision medicine in the early stages of HCC [130].

Innovative microfluidic base materials could reduce the cost of microfluidic technology for POCT applications. To achieve this, microfluidic paper-based analytical devices (μPADs) can be fabricated using nitrocellulose (NC) membranes and screen-printing polyurethane acrylate (PUA) as a barrier material for targeted flow paths and reaction zones. This approach ensures a robust resistance to various solvents, including organic solvents [131]. Ultimately, the authors successfully validated the simultaneous determination of AFP and CEA as low as 136 pg/mL and 174 pg/mL, increasing the potential of microfluidics for early HCC diagnosis in resource-poor areas. Microfluidic POCT devices can employ microfluidics to isolate CTCs. This isolation process can be achieved through two primary strategies. The first strategy capitalizes on the differential affinities between specific antigens present on the surface of the CTCs and specific antibodies present on the surface of the microfluidic device. The second strategy involves the capture of CTCs based on their biophysical properties. Filtration-based microfluidic platforms encompass microfluidic platforms equipped with 3D microfilters or microcolumns. Capturing CTCs from early-stage hepatocellular carcinoma patients using a single antibody may not be sufficient due to their heterogeneity. We prefer isolating CTCs using microfluidic platforms for better results. On the other hand, hydrodynamics-based microfluidic platforms involve the integration of DLD separation technology. Ma et al. [119] conducted a study wherein they integrated microfluidics with multiple sensors to enhance the efficacy of detection. To accomplish this integration, a microfluidic platform was used in conjunction with multifunctional nanosurfaces. These included gold nanoparticles on porous AAO in the SERS substrate and AuMBA@Ag core-shell nanoparticles in the SERS nano-tagging. The application of engineered terahertz (THz) metamaterials in detecting HCC is explored, wherein the THz frequency can be adjusted to align with the vibrational frequency of biomarker molecules associated with liver cancer. This adjustment enhances the sensitivity of HCC detection [132].

Utilizing a microfluidic multichannel network facilitates the acquisition of high throughput measurements and necessitates sample volumes within the microlitre and nanolitre range. Incorporating a microfluidic platform into a POCT device for the early-stage diagnosis of liver cancer enables the concurrent detection of numerous biomarkers, reducing medical expenses for patients and enhancing detection efficacy. Subsequent iterations of microfluidic chips are anticipated to be seamlessly integrated with smartphones and the internet.

4.2.2. Microarray

Researchers primarily use microarray platforms to find new early liver cancer biomarkers. A new lectin microarray platform can specifically detect glycosylation-modified protein biomarkers, allowing for accurate patient diagnosis. The swift advancement of microarray platforms, predominantly including DNA microarrays and protein microarrays, has been noteworthy. Whether it is a DNA microarray or a protein microarray, the high variability of data quality during the assay process and the fabrication of highly specific probes can affect the results. So, the label-free microarray platform integrated with DL is the future trend of multiple pathways for early liver cancer diagnosis.

In the early detection of HCC, numerous genes experience undirected mutations. To facilitate the identification of HCC biomarkers, the microarray platform is employed to detect loss or mutation in fluorescently labeled target DNA. Additionally, the accuracy of the study can be enhanced by incorporating cluster analysis, deep learning, machine algorithms (ML), and feature selection (FS). This approach helps to mitigate the challenges caused by disorder in raw gene expression data and the presence of intense background noise that is commonly associated with early HCC detection [133,134]. Lai et al. [135] conducted a study wherein they designed and implemented a deep neural network (DNN) to extract high-level and feature-compatible abstractions from extensive databases. The performance of this DNN was found to be comparable to that of DL. In early HCC diagnosis, the combination of microarrays and DNNs is mainly applied to find and train new markers for predicting early HCC and to generate predictive models. He et al. [136] then used relative expression ordering and maximum redundancy correlation to find 11 gene pairs capable of distinguishing HCC from non-HCC or adjacent non-cancerous tissues in microarray data generated from 1091 HCC samples and 242 non-HCC samples. Glycosylation modifications of HCC target proteins are closely linked to the initiation of hepatocellular carcinoma. In this way, the emergence of lectin microarrays could provide a platform for the detection of specifically modified glycoproteins in the early HCC process. Zheng et al. [137] then used a microarray of 37 lectins in combination with DNN to ultimately identify 22 salivary glycoproteins that could effectively differentiate between patients with HC and HCC in 118 saliva samples.

In the early detection of HCC through POCT, significant progress has been made in the development of microarray devices. Chi et al. [138] introduced a novel approach involving a protein-labeled, fully printed photonic crystal microarray. This technique utilizes pairs of nanobodies as probes to identify specific protein biomarkers, which can be rapidly detected in blood samples obtained from the fingertip. Label-free microarray platforms, such as those employing reflectance interference spectroscopy and surface plasmon resonance, present the advantage of multiplexing and detecting multiple biomarkers. These platforms significantly improve the efficacy of early HCC detection. Nuria et al. [139] introduced an innovative optical phase-sensitive interferometric biosensor that utilized a label-free microarray configuration, demonstrating its capability to promptly and rapidly identify multiple biomarkers. The integration of label-free microarray platforms with deep learning represents a promising direction for advancing early liver cancer diagnosis.

Microarray platforms depend on extensive experimental instrumentation, which can be enhanced through integration with smartphones. This integration not only decreases testing time but also streamlines the process of POCT, resulting in reduced turnaround time for field test results. Additionally, smartphones offer on-board processing capabilities for efficient data sharing. However, the development of protein microarray systems is still in its early stages due to the inherent challenges associated with the biological activity, stability, and susceptibility to the degradation and precipitation of proteins in vitro assays. To overcome these obstacles and create POCT-compliant microarray platforms, the production of high-quality antibodies or nucleic acid ligands is crucial.

4.2.3. Lateral flow

Lateral flow, also referred to as colloidal gold technology, is the prevailing method utilized in everyday life. This technique relies on the interaction between antibodies and antigens in an immunoreaction. Detection principles encompass indirect, double-antibody sandwich and capture techniques [140]. Lateral flow immunochromatography plays a crucial role in the identification of epidemics and disease surveillance. Owing to its convenient sample sourcing methods (such as finger blood, saliva, nasopharyngeal swabs, etc.) and the presence of specialized sample pads that facilitate swift, cost-effective processing [141].

Currently, most lateral flow immunoassays are limited to qualitative analyses, which poses a limitation for the detection of early-stage HCC. This research can be complemented by the integration of microarray platforms to enhance multiplexing capabilities, the utilization of novel nanoparticles (NPs) and nanomaterials as labels, the adoption of innovative detection methods for signal identification, and the incorporation of smartphones for real-time detection [141]. Liu et al. [142] formed novel Au@Si nanocomposites by coating many gold nanoparticles on silica nanoparticles, and Au@Si produces higher colored signals on FLA for more sensitive detection of miRNA-21. Zhao et al. [143] simultaneously synthesized nanocomposites of AgPd targeting AFP and glycoconjugate (GCA) antibodies, which significantly improved visual detection efficiency.

Furthermore, alongside the utilization of gold nanoparticles for antibody labeling, there is ongoing research and development of innovative nanomaterials. Notably, magnetic particles can serve as colored labels to enhance the sensitivity of detection. Additionally, upconverting phosphors exhibit strong absorption solely in the infrared region and possess facile production methods. Huang et al. [144] evaluated the expression of two proteins, AFP and PIVKA-II, in HCC clinical patients using up-conversion phosphor-FLA. Upconverted phosphor has higher sensitivity and stability than colloidal gold. Moreover, semiconducting materials like quantum dots exhibit fluorescent characteristics and can be readily integrated with biomolecules, enzymes, carbon nanotubes, and colloidal carbon [145]. To enhance the efficacy of HCC biomarker detection, the integration of lateral flow immunochromatography with electrochemical or SERS detection technology was undertaken [146]. To achieve a simultaneous diagnosis of early HCC by AFP in combination with CTC, Chen et al. [147] first purified and functionalized AFP and CTC by the self-developed binding antigen-capture polymerase chain reaction. Final dual detection using LFA and electrochemistry yielded CTC as low as five cells/mL and AFP as low as 5 μg/L. To address the issue of time-consuming SERS scanning of the side-stream immune TL region, Tran's research group

[148] devised a portable SERS reader that employs a fiber-optic probe in conjunction with a nano-diode laser, enabling rapid test result acquisition within seconds. The innovative SERS detector and electrochemical assay offer the opportunity for clinically economical and rapid POCT, enhancing the sensitivity of early HCC diagnosis.

Lateral flow immunochromatography can identify a diverse array of substances without requiring pre-treatment of liquid samples, including blood and saliva. Nevertheless, the detection limit of flow immunoassay is typically elevated. To effectively employ lateral flow for precise early liver cancer diagnosis, advancements are required in its labeling materials, such as carbon nanomaterials, quantum dots, and up-conversion fluorescent materials, among others. These materials can detect multiple targets and enhance the detection sensitivity. Furthermore, in comparison to conventional antibodies, aptamers, and nano-bodies, which are novel biometric components, exhibit compact dimensions and enhanced stability, thereby presenting promising prospects for utilization in biomarker analysis.

4.2.4. Smartphone

The proliferation of smartphones has increased the potential for patients to personalize their healthcare. We can share the results of tests with our physicians at any time to assist in diagnosis, while the high-resolution camera and smart algorithms of smartphones can amplify the test signals and automatically translate them into values or results.

The rapid advancement of smartphones, smartbands, and applications has led to the integration of various technologies and platforms, such as microfluidics, electrochemistry, and SERS. These advancements are facilitated by the highly analytical algorithms and process control capabilities of the computer chip, together with the optical sensing and imaging capabilities of the built-in camera and flash. Additionally, the storage and transmission capabilities of these devices enable the remote sharing of medical test results with a physician through a cloud-based server, enhancing the sensitivity and accuracy of the reader [149]. This integration of technologies and platforms has significant implications for the field of healthcare.

The utilization of smartphone-based in-built flash and camera, coupled with in-built imaging and optical sensing capabilities, enhances the amplification and visualization of the detection signal. This technology finds extensive applications in colorimetric imaging and fluorescence imaging. Lin and colleagues researched a colorimetric approach for antibiotic detection. They utilize digital images captured by smartphones and analyze fluorescent images through a dedicated mobile application [150]. In the identification of the initial phase of HCC, Eda et al. [151] employed the early HCC biomarker AFP as a diagnostic indicator. They fabricated a surface on the NC substrate consisting of spot-shaped AuNPs conjugated with antibodies specific to AFP. The detection of early HCC was accomplished by analyzing the colorimetric characteristics of the AuNPs through smartphone images and utilizing color analysis software. The integration of various technologies, platforms, and components has the potential to enhance the efficacy of early HCC detection greatly. Brittany et al. [152] devised a novel approach for detecting total bilirubin, a promising biomarker for liver cancer, by creating a smartphone-based device utilizing absorbent paper and an acrylic skeleton. This device facilitated the deposition of biochemical reactions onto the paper substrate, enabling the completion of the reaction. By employing a smartphone, the researchers were able to capture and analyze images of colorimetric changes on the mat, subsequently converting them into a consistent bilirubin value. Wang et al. [153] have successfully designed and implemented an optical biosensor for a multichannel smartphone spectrometer (MSS). This innovative system combines a customized smartphone multiview application responsible for controlling optical sensing operations with an optical assembly securely attached to a 3D-printed base. The biosensor can conduct spectroscopic measurements by detecting changes in optical intensity at specific wavelengths or optical spectral excursions.

The ongoing development of AI has the potential to bring about a significant transformation in POCT devices through the integration of cloud-based DL and smartphones. The computational capabilities of smartphones, facilitated by their built-in sensors, diverse software, and applications employing distinctive algorithms, enable them to function as independent sensors for mobile early-stage liver cancer diagnostic POCT. The forthcoming trajectory entails the wireless transmission of hospital information systems to smartphones, encompassing fundamental data pertaining to patients diagnosed with early-stage liver cancer, test outcomes, and medical practitioner input.

4.2.5. Paper-based

The development of paper-based platforms can be used as a substrate for detection platforms such as microfluidics and microarrays, greatly reducing their design costs. Paper-based substrates possess several advantageous characteristics, such as ease of production and modification, exceptional biocompatibility and degradability, and extensive integration into colorimetric, fluorescent, SERS, and other assays. Among these substrates, the paper-based microfluidic μPAD is the most frequently utilized. Additionally, various chemical reactions can be employed to attach surface gold nanoparticles, antibodies, and other entities, thereby achieving a heightened density and uniform distribution of nanostructures [154]. Direct paper-based sandwich immunoassays allow the immobilization of antibodies or nucleic acids by using special nanomaterials to modify the paper base. For antibody-functionalized paper substrates: Liu et al. [155] used graphene oxide and pyrene derivatives to modify paper substrates to provide sufficient carboxyl groups for electrostatic adsorption and EDC coupling of AFP monoclonal antibodies. For the detection of nucleic acid-functionalized paper bases, Shen et al. [156] detected the specific recognition of miRNA-122a, a marker for early HCC diagnosis, using p19 by modifying the p19 protein on a single-walled carbon nanotube-functionalized paper base material.

Ma's team achieved the integration of paper bases into colorimetric and fluorescent photodetection methods [157]. They developed a paper base using highly porous poly (L-lactic) acid nanofibre membranes (p-PLLA) to detect AFP, which allows for the detection of both colorimetric and fluorescent signals, enabling dual-signal detection. The p-PLLA platform incorporates sandwich assays, specifically the sequential arrangement of capture antibody, target molecule, and AuNP-labeled detector antibody. The colorimetric signal was generated by the deposition of red dots produced by AuNP. Following the immunoreaction of p-PLLA with lgG-FITC, the

binding of the detector antibody to lgG-FITC led to a modulation in the intensity of the fluorescence signal. The p-PLLA platform demonstrated an LOD of 0.17 pg/ml in detecting the early liver cancer marker AFP. An additional sandwich-type immunoassay, reliant on paper as the substrate, has been developed to detect AFP. In this method, primary antibodies are immobilized on a paper base using chitosan, enabling the capture of the AFP marker. Following this, a hybridization chain reaction is initiated by modified initiator DNA on the secondary antibody, resulting in the amplification of the fluorescent signal associated with AFP [158]. Maryam's team [159] successfully achieved electrical detection of AFP by integrating paper-based immunoreactivity into an electrochemical detection approach. This was accomplished through the utilization of a novel peptide-modified plastic paper microfluidic chip. The team employed silver-20 wt% graphene nanocomposites to modify the surface electrodes of the paper-based system and nanomodified surface antibodies were enhanced with diphenylpropionic acid (FF) peptides. These modifications were implemented to improve the chip's sensitivity and signal-to-noise ratio.

Paper-based materials are cheap and readily accessible, offering the notable benefits of high flexibility and biodegradability. Moreover, following chemical modifications, their utilization as a chip base finds extensive application in diverse sensor systems. To effectively detect and process target compound molecules, it is imperative to prioritize the analysis of the sample's flow trajectory within the paper, as neglecting this aspect may lead to localized concentration inhomogeneities. In the future, using paper-based sensors will continue to necessitate the utilization of innovative materials arranged in a pattern on paper sheets. When incorporating microfluidic paper-based multiplexing, considerable consideration is given to the propagation of signals in adjacent panels [160].

4.2.6. Instrument free

Although various POCT platforms streamline test operations and integrate systems, their effectiveness is still contingent upon the cost of instruments and the need for continuous maintenance. Moreover, in certain Low Resource Setting (LRS) sites, the utilization of POCT equipment can be hindered by insufficient training and infrastructure configuration [161]. Consequently, instrument-free POCT devices prove to be more appropriate for implementation in LRS settings.

With the widespread implementation of isothermal amplification technology, there has been a significant advancement in the development of instrument-free POCT devices for detecting RNA and other nucleic acid-based markers. In the case of μPADs, which operate without instruments or batteries, the analysis of nucleic acid-based biomarkers typically involves capturing and processing images using a smartphone for color digitizing analysis. An illustrative instance is the development of a multiplexed autonomous disposable nucleic acid amplification test (MAD NAAT) platform by Lisa K's team [162]. This platform, relying on a two-dimensional paper network, effectively diminishes the number of equipment components and associated expenses. The MAD NAAT device securely stores all reagents in a desiccated state, enabling prompt detection without requiring external instruments or interventions. Consequently, the target sample can be processed and identified, ultimately resulting in a discernible detection line that can be observed either through unaided human vision or mobile phone imaging. In a similar vein, Paul's research team has successfully devised an instrument-free nucleic acid amplification heater, known as NINA, which operates on the principles of exothermic chemical reactions and engineered phase change materials. This device demonstrates performance on par with PCR instruments [163]. Furthermore, a comparable methodology has been employed in the development of a disposable chemically heated assay for rapid detection of the H1N1 virus [164]. Furthermore, the utilization of microfluidic platforms enables the absence of instruments. At the same time, isothermal amplification techniques such as dual-pipeline approaches (which involve integrated nucleic acid amplification and plasma gold nanoparticle sensors), integration of customized chain displacement probes, and ring-mediated isothermal amplification have also been employed. These instrument-free testing platforms significantly enhance the practicality of nucleic acid biomarker testing for the early detection of liver cancer.

Instrument-free POCT devices have been found to have potential applications in detecting various biomarkers. For instance, in a study conducted by Chen et al. [165], the detection of hepatocellular carcinoma tumor cells SMMC-7721 was achieved using folate receptor (FR) as a marker and probe, employing dual enzyme-assisted amplification for homogeneous fluorescence. Additionally, the authors demonstrated the feasibility of instrument-free and cost-effective 2D visual inspection of SMMC-7721 on test paper by utilizing CdTe QD and inkjet printing technology. Furthermore, Xu et al. [166] successfully implemented a plasma separation module and a detection module to detect total bilirubin (TBil) and direct bilirubin (DBil) in whole blood to identify early hepatobiliary disease markers. This detection method utilized a mobile phone's ambient light sensor (ALS) to capture colorimetric signals, resulting in a highly sensitive and rapid analysis without the need for specialized instruments. Given their affordability and ease of use, instrument-free detection platforms are expected to be the primary focus of the future development of diagnostic equipment for early-stage liver cancer in the LRS region.

The advancement of point-of-care testing (POCT) devices that do not require instruments is closely intertwined with the incorporation of microfluidics, bioluminescence technology, and smartphones. These devices must possess characteristics such as affordability, user-friendliness, and high performance to cater to the requirements of the LRS region effectively. Given the intended usage in the LRS area, careful consideration should be given to aspects such as sample collection, handling, waste disposal, and biosecurity. The assessment of the equipment's reproducibility and stability in the final examination should consider the environmental and situational factors at the customer's site. Additionally, evaluating the user's proficiency in operating the equipment is crucial for optimizing its performance.

We compare the advantages and disadvantages of various POCT platforms (Table 6). Each route of administration has pros and cons. Paper-based and instrument-free platforms are easy and convenient to operate, which are ideal for families who live where access to clinical services is poor. Microfluidics and microarray can achieve fast and high-throughput detection of biomarkers for early liver cancer, with the potential to be used in central labs. The choice of route of administration depends on many factors, such as the type of

Table 6
Characteristics of the POCT platform for early HCC detection.

Platform	Costs	Advantages	Defect	Clinical application	Target	Diagnosis Performance	Ref
Microfluidics	High	1 Low sample volume and reagent consumption 2 High throughput 3 Small size and highly integrated for instrument miniaturization 4 Reduction in the volume of chemical waste	1 Interference of physical or chemical factors (choice of material, surface roughness) 2 Highly specialized and does not meet the need for user-friendly adaptation 3 Unstable cell viability within the chip	Multiplexed microfluidic paper-based enzyme-linked immunosorbent assay	AFP, CEA	LOD for AFP: 136 pg/mL, LOD for CEA: 174 pg/mL	[132]
Microarray	High	1 Multiplex 2 High sensitivity 3 High specificity	1 Limited detection range: only known DNA and proteins can be analyzed 2 Complex data processing and low accuracy 3 Low specificity probe proteins and limitations of the substrate itself	Lectin microarrays intelligent algorithms	salivary glycoprotein	AUC for the HCC model: 0.886	[138]
Lateral flow	Low	1 Easy to operate, results are visible to the naked eye 2 Capable of high-volume production with long shelf life 3 High specificity, stability and reproducibility	1 Sample pre-treatment has an impact on the results 2 Inaccurate spiking volume and membrane pore structure lead to poor sensitivity and accuracy 3 High quality requirements for labelling materials and antibodies	Lateral chromatography based on lectins	AFP-L3	LOD: 0.8 ng/mL, RSD: 5.2%–8.7 %	169
Smartphone	Medium	1 Integrated artificial intelligence and highly algorithmic to analyze and process detection results and give user feedback 2 Optical sensing with built-in camera and flash promotes imaging capabilities 3 Test data can be transferred and shared with hospitals and physicians	1 DL requires advanced algorithms and data processing networks 2 Smartphones are not widely available in low-resource areas 3 Safety of testing data sharing still needs to be examined	Multi-channel only handpiece with integrated 3D printing mount, optics and multi-view application	Human interleukin-6	LOD: 10.6 pg/mL	[155]
Paper-based	Extremely low	1 Eco-friendly 2 Easy and convenient to operate 3 The paper surface is easy to chemically modify 4 Wide selection of paper-based materials 5 Good biocompatibility and adsorption properties	1 Poor accuracy and consistency 2 Inadequate manufacturing technology 3 Not suitable for electrochemical detection	Paper-based single-walled carbon nanotube (SWNT) field effect transistors (FETs) for paper-based sensors	miRNA-122a	LOD: 0.1 aM	[158]
Instrument Free	Extremely low	1 No special instrumentation required 2 Convenient and easy to use compared to other platforms	1 Requires special fluorescent labels and probes for visual or mobile phone detection 2 Microarrays for detecting nucleic acids are complex and expensive	Homogeneous two-dimensional visual and fluorescence analysis	CTCs	LOD: 0.25 cells/mL	[167]

biomarkers, the properties of the target, and the precision and accuracy required for clinical use.

Clinical methods for detecting cancer biomarkers typically involve ELISA or dry enzyme-labeled electroluminescence after blood extraction, but these methods can be time-consuming. Integrating SERS or SPR with microfluidics allows for the simultaneous detection of multiple proteins and nucleic acids. At the same time, instrument-free PCR amplification and nanomaterial labeling enable rapid and cost-effective detection of early liver cancer markers. Additionally, smartphones can aid in quantitative detection results in colorimetric assays. The development of POCT for early HCC diagnosis should focus not only on validating samples but also on regulating and standardizing clinical application, personnel requirements, quality control, and result analysis.

5. Conclusions

Despite the discovery and validation of new biomarkers for early-stage hepatocellular carcinoma, clinicians still rely on the AFP/AFP-L3 ultrasound diagnosis due to the lack of diagnostic efficacy of a single AFP. More research is needed to bridge the gap between laboratory validation and clinical approval. Early-stage hepatocellular carcinoma processes are complex, and quantitative protein-based biomarker detection is a focus of research, but glycosylation or phosphorylation modifications on their surfaces change dynamically with hepatocellular carcinoma progression. Therefore, to achieve an accurate diagnosis, it is important to detect the dynamic modifying effects on the surface of the protein class and perform a quantitative examination of the protein class.

The enhancement of biomarker development and the creation of novel POCT devices are imperative for the precise identification of early HCC. While the detection of nucleic acid markers in POCT has achieved maturity through the utilization of advanced amplification techniques and templates, the field of protein detection needs more accurate platforms and technologies. Due to the limited detection capabilities of protein in comparison to PCR technology, as well as the potential decrease in stability during the detection process, there is a need to innovate the POCT platform to enable the detection of protein markers.

POCT devices offer a method for detecting cancer biomarkers to assess cancer prognosis, monitor disease progression, and predict outcomes in liver cancer. AFP serves as a crucial marker for early-stage hepatocellular carcinoma diagnosis and plays a significant role in prognostic evaluation. Utilizing converting enzyme nanotags or up-conversion phosphors for AFP, either alone or in conjunction with other prognostic markers, has been proposed for predicting postoperative chemotherapy outcomes in liver cancer patients. Patients diagnosed with liver cancer may experience advantages from POCT for detection and the creation of accurate postoperative survival nomograms to forecast overall survival rates.

Before implementing POCT for early-stage hepatocellular carcinoma, it is crucial to assess test quality, personnel suitability, procedure standardization, costs, and clinician involvement. POCT regulations and standards are established in most developed European countries, with the UK's MHRA proposing a regulatory framework for POCT manufacturing. Successful POCT implementation relies on clinician participation. The UK regulator, the Medicines and Healthcare Products Regulatory Agency (MHRA) has proposed a regulatory framework designed for the manufacture of POCT. Still, the successful implementation of POCT requires that clinicians and the College of Laboratory Clinicians work together to prepare guidelines for POCT.

When developing POCT devices for early liver cancer diagnosis, it is important to validate and implement them carefully to ensure they produce accurate results comparable to laboratory tests. Involving clinicians and laboratory personnel in the initial R&D validation process can provide a better understanding of current approaches to care in the face of clinical challenges. At the stage of technology selection and development, it is important to consider costs and assess the ratio between the target population and the environmental benefits to determine their acceptability. The launch of the final POCT device must be subject to a rigorous external quality assessment and an internal quality control system (user training and frequency of testing, review of test coverage and results, etc.). Ultimately, to achieve accurate identification of early HCC on a patient-individualized basis, the data must be merged or exchanged with the hospital medical record, and AI will be integrated into POCT for outcome analysis and diagnosis. Ultimately, to achieve accurate identification of early HCC on a patient-individualized basis, the data must be merged or exchanged with the hospital medical record, and AI will be integrated into POCT for outcome analysis and diagnosis.

Availability of data and materials

Data sharing does not apply to this article as no datasets were generated or analyzed during the current study.

CRediT authorship contribution statement

Mengxiang Liu: Writing – original draft. **Yanrong Wen:** Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

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